INDUCED AVOIDANCE BEHAVIOUR TO LIGHT IN THE PROTOZOA TETRAHYMENA

BY

STEN R. BERGSTRÖM

UPPSALA 1969
ALMQVIST & WIKSELLS BOKTRYCKERI AB

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548912_0033

INDUCED AVOIDANCE BEHAVIOUR TO LIGHT IN THE PROTOZOA TETRAHYMENA

INAUGURAL DISSERTATION

BY

STEN R. BERGSTRÖM
Fil. lic., Sthlm

by due permission of the faculty
of social sciences of the University of Uppsala,
to be publicly discussed in lecture room IV,
on May 21st, 1969, at 10 a.m.
for the degree of doctor of philosophy

UPPSALA 1969
ALMQVIST & WIKSELLS BOKTRYCKERI AB

INDUCED AVOIDANCE BEHAVIOUR TO LIGHT IN THE PROTOZOA TETRAHYMENA

BY

STEN R. BERGSTRÖM

UPPSALA 1969

UNIV.
LEIDEN
BIBL.

This paper is based on the following studies, which will be referred to by citation of the appropriate Roman numerals:

I. BERGSTRÖM, S. R. (1968*a*). Induced avoidance behaviour in the Protozoa Tetrahymena. *Scand. J. Psychol.*, *9*, 215–219.

II. BERGSTRÖM, S. R. (1968*b*). Acquisition of an avoidance reaction to light in the Protozoa Tetrahymena. *Scand. J. Psychol.*, *9*, 220–224.

III. BERGSTRÖM, S. R. (1969a). Amount of induced avoidance behaviour to light in the Protozoa Tetrahymena as a function of time after training and cell fission. *Scand. J. Psychol.*, *10*. 16–20.

IV. BERGSTRÖM, S. R. (1969*b*). Avoidance behaviour to light in the Protozoa Tetrahymena. The effect of a gradual versus an abrupt boundary between dark and light. *Scand. J. Psychol.*, *10*, Preprint.

The investigations were performed at the Department of Psychology, University of Uppsala, Sweden. I wish to express my deep gratitude to Professor Gunnar Johansson, the head of the department, for his continuous interest, valuable comments, and generous support. He has also kindly offered his time to read the manuscripts.

I am greatly indebted to Fil.lic. Martin Lindberg, for his valuable advice concerning the handling of the Tetrahymena and for supplying the subjects.

My special thanks are directed to Fil.kand. Kajsa Svahn for discussions and constructive criticism regarding the reporting of the results.

Fil.mag. Cajsa Rumar has revised the English language, Mrs. Eva-Brita Skarby has typewritten the manuscripts, and Fil.kand. Karl-Erik Sundström has drawn the figures. To these persons, my warm thanks.

Finally, I wish to express my gratitude to my family, and to my friends and colleagues at the Department of Psychology, Uppsala, for their interest and encouraging attitudes during the work.

The investigations were supported by grants from the Swedish Council for Social Science Research.

INTRODUCTION

Since Smith (1908) reported that Paramecia could by practice increase their ability to turn around in a constricting tube, many investigations, claiming to have demonstrated primitive 'learning' in Protozoa, have been published. The general aim of these investigations was of course to extend the knowledge about the capacity of the living organisms, but it seemed also that many investigators were intrigued by the possibility of studying learning in a single cell, i.e. within a Protozoa. But in almost every case, it has been possible to show that the reported

behaviour modifications could be attributed to factors other than learning (Jensen, 1964, Thorpe, 1964). Despite the fact that the crucial proof of the capacity or incapacity for learning in acellular organisms was still lacking, the interest in the problem has decreased remarkably during the last ten years. This was probably due to many facts, e.g.: the results hitherto were not encouraging; it is extremely difficult to carry out and to interpret conditioning experiments with Protozoa; the psychology of the Protozoa may yet seem to be of peripherical interest.

The problem is, however, actualized by the growing interest in the biochemical basis of learning and memory (See Richter, 1966, for references). The view that memory can be coded at the molecular level has given rise to claims to very advanced biochemical technics. The recent marvellous progress in the knowledge about fundamental biological phenomena in general, has very often been results of such advanced technics applied to the most primitive organisms, where these phenomena appear relatively accessible for investigation. This technic must be applicable even to experiments concerning the molecular code of memory. Hence, it is now highly relevant to search for learning and memory on as low a level of evolution as possible.

Earlier experiments show that if 'learned' behaviour might exist on the protozoan level, it would be rather difficult to distinguish from the spontaneous activities of the animals. The general idea of the present investigations was to applicate mass technic and extensive use of various control groups, in order to make even very small behaviour variations discernible. In the first place, the aim of the experiments was to investigate if such a technic might throw some light upon the old but unanswered question about learning in Protozoa, but ultimately the goal was to find an appropriate subject for the scientists, working with the biochemistry of learning and memory.

METHOD

A method to induce a behaviour modification (training), and an appropriate method to observe it (testing) were the two major methodological problems in the present studies.

Training.

In each of the four studies (I–IV), groups of Tetrahymena pyriformis, which normally do not react to light but give avoidance reactions to electric shocks, were trained to avoid light by exposures to repeated presentations of light stimuli and electric shocks paired. Control groups received either shocks or light stimuli or neither of the two stimuli. In study II–IV cultures dividing synchronously were used, in order to avoid the disadvantages of having uncontrolled cell fissions during the experiment.

Pilot studies indicated that a wide range of light intensities and shock intensities respectively gave no systematic variations on the observable behaviour of the Tetrahymena. Hence, the precise choice of stimulus intensities was not a central problem, and practical reasons could therefore to some extent be the guiding factors.

The voltage of the shocks was chosen to cause strong avoidance reactions, but the animals could stand up to 6 times the used voltage before any lasting effects could be observed. The amount of electric stimulation received by a single animal was proportional to the conductiv-

ity of the animal relative to the surrounding fluid and to that section face of the animal being orthogonally exposed to the current flow. Since a Tetrahymena only measures about $30 \times 50\mu$, a single animal received a very small share of the total current flow through the training chamber. Alternating current was used in order to avoid the effects of polarized animals and of galvano-taxis.

Testing

In order to test the degree of avoidance behaviour, samples were drawn from the experimental groups and from the control groups and placed in chambers, lighted through a screen which gave rise to lighted and shaded zones in the chambers. If the animals were indifferent to light, the population density should be the same in the lighted and in the shaded zones. If the animals did avoid light, the population density should be lower in the light than in the dark. In study I–III, the test chambers contained 15 lighted zones, methodically arranged all over the chamber, the boundaries between lighted and shaded zones were abrupt, and the animals were randomly distributed over the chamber at the beginning of the testing. In study IV, the test chamber contained only one lighted and one shaded zone, both gradual and abrupt boundaries between lighted and shaded zones were used, and the animals were placed in the shaded zone at the beginning of the testing.

The frequency of animals in the lighted and in the shaded zones, respectively, was observed by photographing the test chamber at fixed time intervals.

RESULTS

The main results reported in the four papers are in summary:

I. After 15 stimulus presentations there was in the experimental groups a successive decrease of the number of animals in the lighted zones during the first $3\frac{1}{2}$ minutes of the test period, i.e. the animals had acquired a tendency to avoid the lighted zones. No such tendency was found in the control groups.

II. With increasing number of stimulus presentations, there was an increase in the tendency of the animals in the experimental groups to avoid the lighted zones. More than about 30 stimulus presentations, however, did not seem to yield any appreciable contribution to the avoidance tendency.

III. (a) In the absence of training, the acquired avoidance tendency decreased. After 30 stimulus presentations, it took somewhat more than 2 hours for the tendency to cease. (b) A cell division during the testing did not influence the course of the decreasing avoidance tendency.

IV. In study I–III, where the boundaries between the dark and the lighted zones were fairly abrupt, the avoidance tendency was obvious but far from complete. With a gradual boundary, however, there was after 30 stimulus presentations an almost complete tendency of the experimental group to avoid the lighted zone.

The results of the four studies constantly indicated, that a fairly small number of exposures to light stimuli and electric shocks in combination will induce the Tetrahymena to react to a boundary between dark and light as if it were an obstacle, when approaching it from the dark side, but not when approaching it from the light side. Light stimuli only or electric shocks only did not produce that effect.

In the natural conditions of the Ciliates (and assuredly of many other classes) gradual rather than abrupt environmental changes are the rule—disregarding contacts with solid objects. Accordingly, the avoidance pattern of the Ciliates (IV) is adapted to gradual stimulus changes. The fact that there was such an obvious avoidance behaviour in study I–III, where the boundary between dark and light was abrupt, must to some extent be attributed to the fact that the boundary was not perfectly abrupt, owing to light refraction on the edges of the screens and in the test chambers.

The effectiveness of the avoidance reaction is in general depending on the intensity of the avoidance-eliciting stimulation: increasing stimulus intensity leads to increasing deviation from the primary course of the animal (IV). The increasing avoidance tendency with increasing number of stimulus presentations (II) and the decreasing avoidance tendency in the absence of training (III) are probably due to increasing respectively decreasing course deviations. Should this interpretation be correct—and there seems to be much in favour of it (IV)—increasing amount of training would be equivalent to increasing stimulus intensity in regard to the effect on the course deviation.

It would certainly be too premature to make any extensive argumentation concerning the real biochemical nature of the acquired behaviour modification. There is, however, no reasonable ground to assume that the training causes any alteration of the genetic code of the animal. The induced behaviour then must emanate from modifications of other chemical structures than the genes. In a Tetrahymena, the material of the body is constantly synthesized in accordance with the genetic information of the animal. If the genetic information is unmodified, the new-synthesized material must be of the original type, and then the acquired avoidance tendency ought to wane successively as the modified material is replaced. Fission occurs when the mass of the animal has doubled, thus maintaining an equilibrium between synthesis of new material and fission. The fission involves no recombination of the genes. Hence, there is no reason to expect this process to affect the course of the waning of the acquired behaviour. This argumentation was supported by the results of study III.

The results of the entire series of experiments show that the protozoa Tetrahymena pyriformis has a capacity for establishing a temporary connection between a primarily neutral light stimulus and the avoidance reaction, when exposed to light stimuli combined with avoidance-eliciting electric shocks. The Tetrahymena do not possess a nervous system in the usual sense, and hence this effect can not be attributed to neural connections. Perhaps we here deal with a more or less purely chemical type of learning. Much more research is yet necessary before anything could be known for certain about this matter. At present, however, we can state that a primitive form of 'associative learning' beyound the level of nervous systems has been demonstrated.

The properties of the Tetrahymena are of course of limited interest per se. Tetrahymena is

a fairly high-developed protozoa, and hence perhaps not an optimal subject for biochemical investigations. But the actual findings indicate the possibility and the expediency of searching for learning further down in the evolution in order to find a species still better fitted for a penetrating analysis of the fundamental phenomena in learning and memory.

REFERENCES

JENSEN, D. (1964). Paramecia, Planaria, and pseudo-learning. *Animal behaviour supplement I* (London: Baillière, Tindall & Cassell) 9–20.

RICHTER, D. (1966) *Aspects of Learning and Memory*. London: The Whitefriars Press Ltd Pp. 97–99.

SMITH, S. (1908). The limits of educability of Paramecium. *J. comp. physiol. Psychol.*, 18, 499–510.

THORPE, W. (1964). *Learning and instinct in animals*. London: Methuen Pp. 183–190.